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Stimulation of sensory nerves and CGRP attenuate pancreatic damage in ischemia/reperfusion induced pancreatitis

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A Study Design
B Data Collection
C Statistical Analysis
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Summary

Background: Previous studies have shown that sensory nerves and calcitonin gene-related peptide (CGRP) affect caerulein-induced pancreatitis. The aim of this study was to examine the role of capsaicin-sensitive nerves and the impact of CGRP administration on necrotizing pancreatitis induced by ischemia/reperfusion.

Material/Methods: Ablation of sensory nerves was made by capsaicin 10 days before induction of pancreatitis. Acute pancreatitis was induced in rats by limitation of pancreatic blood flow (PBF) followed by reperfusion. Treatment with saline or CGRP (10 μg/kg s.c.) or stimulation of sensory nerves by low doses of capsaicin (0.5 mg/kg s.c.) was performed 1 h before ischemia. After 1 h reperfusion we examined pancreatic blood flow (PBF), plasma amylase and lipase activity, plasma interleukin-1β (IL-1β) concentration, pancreatic DNA synthesis and morphological signs of pancreatitis.

Results: Ischemia followed by 1 h reperfusion led to induction of necrotizing pancreatitis, manifested by morphological signs of pancreatic damage, decrease in pancreatic DNA synthesis and PBF, as well as an increase in plasma amylase and lipase activity and plasma IL-1β concentration. Both, treatment with CGRP and stimulation of sensory nerves attenuated pancreatic damage. Ablation of sensory nerves enhanced I/R evoked pancreatic damage. The deleterious effect of deactivation of sensory nerves on I/R-induced pancreatitis was partly reversed by administration of CGRP prior to I/R.

Conclusions: Stimulation of sensory nerves protects the pancreas against damage evoked by I/R, whereas ablation of these nerves aggravates tissue damage in the pancreas exposed to I/R. The beneficial effect of sensory nerves is partly dependent on CGRP release.

key words: **capsaicin • sensory nerves • CGRP • acute necrotizing pancreatitis • interleukin-1β**

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BACKGROUND

Primary sensory neurons serve for conduction of nociceptive information to the central nervous system, but also they are able to release neuromediators from activated peripheral endings, a process that is basic for local 'axon reflex' [1]. Sensory fibers have a special sensitivity to capsaicin [2]. Capsaicin, the main pungent ingredient of chili pepper, binds to specific vanilloid (capsaicin) receptors on primary sensory neurons [3,4]. Low doses of capsaicin stimulate primary sensory nerves by opening the nonselective cation channels involved in vanilloid receptors, resulting in local release of neurotransmitters, such as calcitonin gene-related peptide (CGRP) and substance P (SP) [5,6]. On the other hand, high neurotoxic doses of capsaicin lead to ablation of sensory nerves with a decrease in the plasma and tissue level of CGRP [7,8].

In the gut, CGRP immunoreactivity has been found in the nerve fibers innervating the stomach, the intestine and the pancreas [9,10]. Capsaicin-sensitive primary afferent nerves are involved in different aspects of gut pathology. Stimulation of sensory fibers, as well as administration of exogenous CGRP, has been reported to inhibit the formation of lesions in the stomach [11–13], whereas the ablation of sensory nerves aggravates gastric mucosal lesions induced by various ulcerogenic factors, and prolongs gastric ulcer healing [13–15]. A similar influence of capsaicin and CGRP on tissue damage development has been found in the pancreas during induction of acute edematous pancreatitis by caerulein. Activation of sensory nerves [16,17] or treatment with CGRP [18] prior to induction of acute pancreatitis by caerulein attenuates the pancreatic damage, whereas deactivation of sensory nerves contributes to aggravation of acute pancreatitis [16,17].

The protective effects of afferent nerve stimulation or CGRP administration in the stomach and the pancreas have been attributed, at least in part, to the improvement of gastric and pancreatic circulation [14,17–19]. In the pancreas, a vascular mechanism has been shown to play an important role in the maintenance of organ integrity. Microvascular perfusion failure is a characteristic hallmark of experimental and clinical pancreatitis [20–22]. Experimental studies show that ischemia alone may initiate pancreatitis, and always aggravates pancreatic damage [20,23,24], whereas vasodilatation and improvement of pancreatic blood flow inhibit the development of acute pancreatitis [17,18]. Also, early disturbances of pancreatic circulation are observed in acute pancreatitis caused by other, non-vascular factors [17,18,23]. Reduction of blood flow in the pancreatic microcirculation is well known to result in the formation of thrombi in the capillaries, activation of leukocytes, intrapancreatic release and activation of digestive enzymes, and formation of oxygen-derived free radicals and pro-inflammatory cytokines [21].

The aim of our study was to examine the influence of capsaicin-sensitive nerve stimulation or ablation and CGRP administration on the development of necrotizing and hemorrhagic pancreatitis induced by ischemia with reperfusion.

MATERIAL AND METHODS

Our experiments were performed on male Wistar rats weighing 160–180 g, and were conducted in accordance with an experimental protocol approved by the Committee for Research and Animal Ethics at the Jagiellonian University.

Studies were carried out on the following experimental groups:

1. sham-operated animals injected with saline (0.9% NaCl s.c. to serve as a control group; N=15 animals);
2. animals with ischemia-reperfusion induced acute pancreatitis (I/R) (N=10 animals);
3. sham-operated animals with stimulation of sensory nerves by a low dose of capsaicin (0.5 mg/kg s.c.) (N=10 animals);
4. sham-operated animals with ablation of sensory nerves by high neurotoxic dose of capsaicin (100 mg/kg) (N=10 animals);
5. sham-operated animals injected with CGRP (10 μg/kg s.c.) (N=10 animals);
6. sham-operated animals with ablation of sensory nerves by a high neurotoxic dose of capsaicin (100 mg/kg) and injected with CGRP (10 μg/kg s.c.) (N=10 animals);
7. animals with stimulation of sensory nerves by a low dose of capsaicin (0.5 mg/kg s.c) and induction of I/R pancreatitis (N=10 animals);
8. animals with ablation of sensory nerves by high neurotoxic dose of capsaicin (100 mg/kg) and induction of I/R pancreatitis (N=8 animals);
9. animals injected with CGRP (10 μg/kg s.c.) before induction of I/R pancreatitis (N=10 animals);
10. animals with ablation of sensory nerves by a high neurotoxic dose of capsaicin (100 mg/kg) and injecetd with CGRP (10 μg/kg s.c.) before induction of I/R pancreatitis (N=9 animals).

Ablation of afferent sensory nerves was induced by pretreatment with capsaicin (Fluka, Buchs, Switzerland) in a total dose of 100 mg/kg, which was given in six injections (2.5+10+12.5+25+25+25 mg/kg s.c.) over 3 consecutive days. Two injections per day were performed in rats under ether anesthesia, and a recovery period of 10 days was allowed before further experiments. To assess the effectiveness of sensory denervation, the day before the induction of pancreatitis, a drop of capsaicin (0.33 mM) was instilled into the rats' eyes, and animals showing any wiping movements were excluded from the study.

Acute pancreatitis was induced after fasting for 24 h with free access to water. Rats were anesthetized with ketamine (50 mg/kg intraperitoneally, Bioketan, Biowet, Gorzów, Poland). After longitudinal laparotomy, ischemia of the splenic region of the pancreas was induced by clamping the inferior splenic artery for 30 min using microvascular clips. Thirty minutes later, the microvascular clips were removed for reperfusion and

the abdominal cavity was closed. The rats were sacrificed after 1 h reperfusion. In sham-operated animals, longitudinal laparotomy and mobilization of the pancreas was performed without clamping any arteries.

Treatment with saline or CGRP (10 μg/kg s.c.) or stimulation of sensory nerves by low doses of capsaicin (0.5 mg/kg s.c.) were performed 1 h before laparotomy.

Synthetic rat CGRP-I was purchased from Sigma Chemical Co. (St. Louis, Missouri, USA).

Following 1 h reperfusion or 1 h after sham-operation, the animals were anesthetized with ketamine (50 mg/kg i.p, Bioketan, Biowet, Gorzów, Poland) and the abdomen was opened. The pancreas was exposed for the measurement of blood flow by laser Doppler flowmeter using a PeriFlux 4001 Master monitor (Perimed AB, J(rf(lla, Sweden), as previously described elsewhere [25]. The pancreatic blood flow was presented as percent change from the control value obtained in rats infused with saline.

Immediately after pancreatic blood flow had been measured, the abdominal aorta was exposed and blood was taken for plasma amylase, lipase and IL-1β determination. Plasma amylase activity was determined by an enzymatic method (Amylase reagent set - kinetic, Alpha Diagnostic Ltd, Warsaw, Poland]. Plasma lipase activity was determined with a Kodak Ectachem DT II System Analyzer (Eastman Kodak Company, Rochester, New York, USA) using Lipa DT Slides (Vitros DT Chemistry System, Johnson & Johnson Clinical Diagnostics Inc, Rochester, New York, USA). The values of plasma amylase and lipase activity are expressed as units/liter. Plasma IL-1β was measured using the BioSource Cytoscreen rat IL-1β kit based on a solid phase sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) (BioSource International, Camarillo, California, USA). Concentrations are expressed as pg/ml.

After blood withdrawal the pancreas was carefully dissected from its attachment to the stomach, the duodenum and the spleen. Fat and peripancreatic tissue were trimmed away. Samples of pancreatic tissue were collected for study of DNA synthesis and morphological examination. The rate of DNA synthesis in a portion of minced pancreatic tissue was determined by incubating the tissue at 37°C for 45 min. in 2 ml of medium containing 8 μCi /ml of [^{3}H]thymidine ([6-^{3}H]-thymidine, 20–30 Ci/mmol (Institute for Research, Production and Application of Radioisotopes, Prague, Czech Republic). The reaction was stopped with 0.4 M perchloric acid containing the carrier thymidine (5 mM). Tissue samples were centrifuged and the precipitate washed twice in 0.2 M cold perchloric acid and recentrifuged. RNA was hydrolyzed in 0.3 M KOH incubated for 90 min. at 37°C. DNA and protein were reprecipitated with 10% perchloric acid. After standing for 10 min. on ice, the tubes were centrifuged and the supernatant was discarded. DNA in the residual pellets was solubilized in 10% perchloric acid by heating at 70°C for 20 min. The denatured protein was removed by centrifugation for 20 min. Using calf thymus as a standard, the DNA concentration was determined by the Giles and Myers procedure [26]. The incorporation of [^{3}H] thymidine into DNA was determined by counting 0.5 ml DNA-containing supernatant in a liquid scintillation system. DNA synthesis was expressed as [^{3}H] thymidine disintegrations per minute per microgram DNA (dpm/μg DNA).

For histological examination, samples of pancreatic tissue were excised, fixed in 10% formalin, and embedded in paraffin, and sections were stained with hematoxylin and eosin. The slides were examined histologically by two experienced pathologists without knowledge of the treatment given. The histological grading of edema was made using a scale ranging from 0 to 3: 0 =no edema, 1 =interlobular edema, 2 =interlobular and moderate intralobular edema, and 3 =severe interlobular and intralobular edema. Leukocyte infiltration was graded from 0 to 3: 0 =absent, 1 =scarce perivascular infiltration, 2 =moderate perivascular and scarce intralobular infiltration; 3 =abundant diffuse infiltration. Grading of vacuolization was based on the percentage of cells involved: 0 =absent, 1 =less than 25%, 2 =25–50% and 3 =more than 50%. Findings of acinar necrosis were graded: 0 =absent, 1 =less than 15 % of cells involved, 2 =form 15 to 35 % of cells involved, 3 =more than 35% of cells involved. Hemorrhages were graded: 0 =absent, 1 =from 1 to 2 foci per slide, 2 =from 3 to 5 foci per slide, 3 =more than 5 foci per slide.

Statistical analysis

The differences between mean values from various groups of experiments were compared by variance analysis and Student's t-test for unpaired data. A difference with a P value of less than 0.05 was considered statistically significant. Results are expressed as means±S.E.M.

RESULTS

The pancreases of sham-operated saline-infused animals showed no tissue alteration macroscopically or under light microscopy (Table 1). Treatment with a low dose (0.5 mg/kg) or a high dose (100 mg/kg) of capsaicin, or administration of CGRP, also did not affect the morphology of pancreatic tissue (Table 1). The same effect was observed after ablation of sensory nerves combined with administration of stimulatory doses of capsaicin or CGRP in sham-operated animals (Table 1). Pancreatic ischemia followed by 1 h reperfusion (I/R) produced acute necrotizing pancreatitis in all tested rats. Microscopic examination showed severe inter- and intralobular edema accompanied by 3 to 5 foci of hemorrhages per slide. Inflammatory leukocyte infiltration was scarce, predominantly perivascular. Necrosis was observed in all cases of I/R-induced pancreatitis and involved from less than 15% up to 35% of acinar cells. Vacuolization was observed in less than 15% of the acinar cells.

Stimulation of sensory nerves by a low dose of capsaicin (0.5 mg/kg s.c) before induction of I/R-evoked pancreatitis attenuated pancreatic tissue damage (Table 1). Pancreatic edema, hemorrhages, inflammatory infiltrate were less

Table 1. Effect of ischemia/reperfusion (I/R), stimulatory or neurotoxic dose of capsaicin and CGRP applied alone or in combination on the histological manifestation of acute pancreatitis.

		Edema (0–3)	Hemorrhages (0–3)	Infiltration (0–3)	Necrosis (0–3)	Vaculization (0–3)
Control	N=15	0	0	0	0	0
I/R	N=10	2/3	2	1/2	1/2	1
Capsaicin 0.5 mg/kg	N=10	0	0	0	0	0
Capsaicin 100 mg/kg	N=10	0	0	0	0	0
CGRP	N=10	0	0	0	0	0
Capsaicin 100 mg/kg +CGRP	N=10	0	0	0	0	0
Capsaicin 0.5 mg/kg +I/R	N=10	2	0/1	0/1	0	1
Capsaicin 100 mg/kg +I/R	N=8	3	1	1/2	2	1
CGRP + I/R	N=10	2	0/1	1	1	1
Capsaicin 100 mg/kg + CGRP + I/R	N=9	2/3	1	1	0/1	1

Numbers represent the predominant histological grading in each group

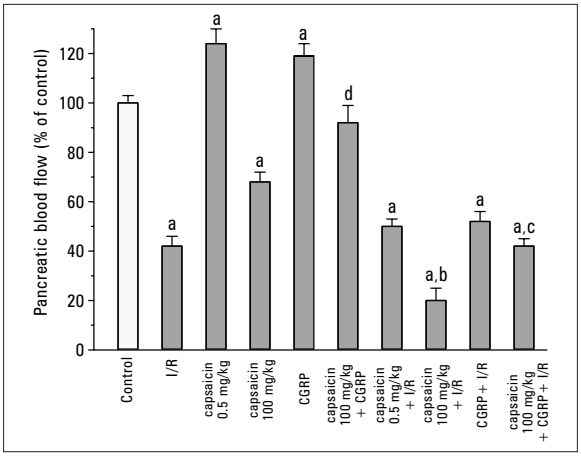

Figure 1. Effect of ischemia/reperfusion (I/R), stimulatory (0.5 mg/kg) or neurotoxic (100 mg/kg) dose of capsaicin and CGRP applied alone or in combination on pancreatic blood flow. Mean±SEM of 8–15 observations in each group. (a) $P<0.05$ compared to control, (b) $P<0.05$ compared to I/R alone, (c) $P<0.05$ compared to I/R combined with neurotoxic dose of capsaicin (100 mg/kg); (d) $P<0.05$ compared to neurotoxic dose of capsaicin (100 mg/kg) given alone.

prominent and no necrosis was observed. Only acinar cell vacuolization was present with the same intensity as in animals without treatment with stimulatory doses of capsaicin before induction of acute pancreatitis (grade 1).

A similar, but less pronounced protective effect was observed in animals treated with CGRP before induction of acute pancreatitis. In this group of animals, pancreatic edema, as well as leukocyte infiltration were predominantly interlobular (grade 2 and 1, respectively). The number of hemorrhages was reduced to 0–2 foci per slide, whereas necrosis involved less than 15% of the acinar cells (Table 1). Treatment with CGRP was without effect on the number of acinar cells, with vacuolization in animals with I/R-induced pancreatitis.

Ablation of sensory nerves (capsaicin 100 mg/kg) enhanced I/R evoked pancreatic damage (Table 1). After 1 h reperfusion, a prominent severe inter- and intralobular edema was present (grade 3). Acinar cell necrosis was seen in all cases, and 15–35% of the cells were involved (grade 2). Inflammatory infiltratation and vacuolization of acinar cells were not affected by ablation of sensory nerves before induction of I/R pancreatitis, whereas the number of hemorrhages was slightly reduced.

Treatment with CGRP before I/R-induced pancreatitis partly reversed the deleterious effect of sensory nerve ablation (Table 1). Pancreatic edema, leukocyte infiltration, and necrosis were reduced. On the other hand, treatment with CGRP before induction of acute pancreatitis in animals with ablation of sensory nerves did not affect the number of hemorrhages and vacuolization of acinar cells.

I/R-induced acute pancreatitis caused a reduction of pancreatic blood flow by 58%, when compared to sham-operated animals ($p<0.0001$) (Figure 1). Stimulation of sensory nerves by a low dose of capsaicin (0.5 mg/kg), as well as administration of CGRP in sham-operated animals, led to an increase in pancreatic blood flow by 24% ($p=0.0003$) and 18% ($p=0.001$), respectively, whereas ablation of sensory nerves by a high dose of capsaicin (100 mg/kg) caused a decrease in pancreatic blood flow by 32% ($p<0.0001$). In animals with ablation of sensory nerves, administration of CGRP almost completely reversed the capsaicin-induced reduction in pancreatic blood flow ($p=0.0069$). In animals with I/R-induced pancreatitis, pretreatment with CGRP or a low dose of capsaicin (0.5 mg/kg) caused a weak increase in pancreatic blood flow, but this effect was statistically insignificant (Figure 1). Ablation of sensory nerves by high doses of capsaicin (100 mg/kg) led to an additional and significant reduction in pancreatic blood flow in animals with I/R-induced pancreatitis ($p=0.0014$). This effect was significantly reversed by pretreatment with CGRP ($p=0.004$).

In sham operated saline-infused control rats, pancreatic DNA synthesis reached 59.8±1.2 dpm/μg DNA (Figure 2). In animals with I/R-induced pancreatitis, pancreatic DNA synthesis was reduced by 33% ($p<0.0001$). Neither treatment with a stimulatory dose of capsaicin (0.5 mg/kg) nor CGRP administration affected pancreat-

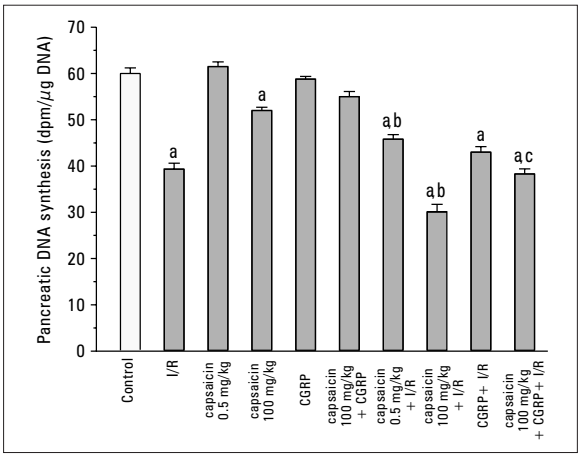

Figure 2. Effect of ischemia/reperfusion (I/R), stimulatory (0.5 mg/kg) or neurotoxic (100 mg/kg) dose of capsaicin and CGRP applied alone or in combination on pancreatic DNA synthesis. Mean±SEM of 8–15 observations in each group. (a) P<0.05 compared to control, (b) P<0.05 compared to I/R alone, (c) P<0.05 compared to I/R combined with neurotoxic dose of capsaicin (100 mg/kg).

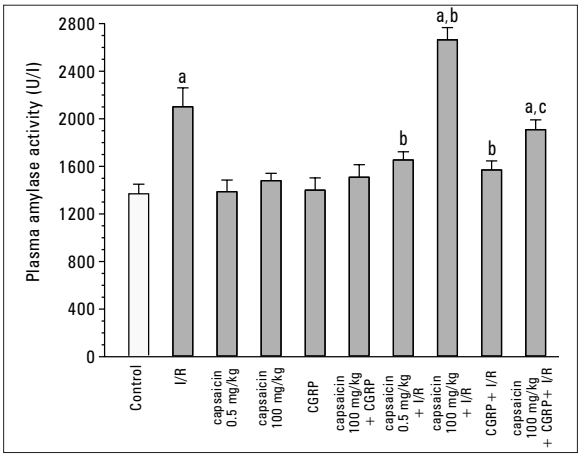

Figure 3. Effect of ischemia/reperfusion (I/R), stimulatory (0.5 mg/kg) or neurotoxic (100 mg/kg) dose of capsaicin and CGRP applied alone or in their combination on plasma amylase activity. Mean±SEM of 8–15 observations in each group. (a) P<0.05 compared to control, (b) P<0.05 compared to I/R alone, (c) P<0.05 compared to I/R combined with neurotoxic dose of capsaicin (100 mg/kg).

ic DNA synthesis in sham-operated animals. On the other hand, ablation of sensory nerves (capsaicin 100 mg/kg) caused a decrease in pancreatic DNA synthesis by 14% in animals without induction of acute pancreatitis ($p<0.0001$). This effect of sensory nerve ablation on pancreatic DNA synthesis was partly reversed by treatment with CGRP. Pretreatment with capsaicin at the dose of 0.5 mg/kg significantly attenuated the reduction in DNA synthesis in rats with I/R-induced pancreatitis ($p=0.0007$). The effect of pretreatment with CGRP was weaker and insignificant. Ablation of sensory nerves (capsaicin 100 mg/kg) in combination with I/R-induced pancreatitis caused a maximal decrease in pancreatic DNA synthesis (30.0±1.7 dpm/μg DNA). In this group of animals, administration of CGRP led to reversion of the drop in DNA synthesis evoked by sensory nerve ablation ($p=0.0018$), which reached a value similar to that observed in I/R-induced pancreatitis without ablation of sensory nerves.

Plasma amylase and lipase activity

Plasma amylase (Figure 3) and lipase (Figure 4) activity in sham-operated control saline-infused rats reached 1358±77 and 54.7±3.9 U/L, respectively. Neither stimulation of sensory nerves (capsaicin 0.5 mg/kg), nor ablation of sensory nerves (capsaicin 100 mg/kg), nor administration of CGRP, nor a combination of a high dose of capsaicin (100 mg/kg) with CGRP affected plasma amylase and lipase activity in sham-operated animals. I/R-induced pancreatitis caused an increase in plasma amylase and lipase activity by 54% ($p=0.002$) and 900% ($p<0.0001$), respectively, when compared with sham-operated control rats. Pretreatment with either a low dose of capsaicin (0.5 mg/kg) or CGRP reduced the I/R evoked increase in plasma amylase and plasma lipase activity by about 25%. Ablation of sensory nerves (capsaicin 100 mg/kg) combined with I/R-induced pancreatitis led to a maximal increase in plasma amylase and lipase activity, reaching 2668±103 U/L and 684.3±27.0 U/L, respectively. Treatment with CGRP before I/R-induced pancreatitis abolished the deleterious effect of sensory nerve ablation on plasma amylase (Figure 3) ($p<0.0001$) and lipase (Figure 4) activity ($p=0.0072$).

In control sham-operated rats, the plasma IL-1β concentration was 73.4±1.9 pg/mL (Figure 5). Treatment with a low dose of capsaicin (0.5 mg/kg) or administration of CGRP had no effect on the plasma IL-1β level in sham-operated rats. Ablation of sensory nerves (capsaicin 100 mg/kg) caused a 40% increase in plasma IL-1β concentration ($p<0.0001$), which was diminished by treatment with CGRP. I/R-induced pancreatitis caused an 84% increase in plasma IL-1β concentration ($p<0.0001$). Stimulation of sensory nerves (capsaicin 0.5 mg/kg) reduced I/R-induced increase in plasma IL-1β concentration by about 32% ($p=0.0138$). Administration of CGRP had no significant effect on plasma IL-1β concentration in animals with I/R-induced pancreatitis. Ablation of sensory nerves (capsaicin 100 mg/kg) in combination with I/R-induced pancreatitis caused a maximal increase in plasma IL-1β concentration, which reach the value of 211.6±8.7 pg/mL. Pretreatment with CGRP before I/R-induced pancreatitis reduced the deleterious effect of sensory nerve ablation on plasma IL-1β concentration ($p=0.0076$).

Discussion

The present study confirms and extends previous findings that stimulation of sensory nerves [16,17] and administration of CGRP [18,27] before and during induction of pancreatitis reduces pancreatic damage in this disease. In our previous study, acute pancreatitis was evoked by stimulation of the pancreas, by an overdose of

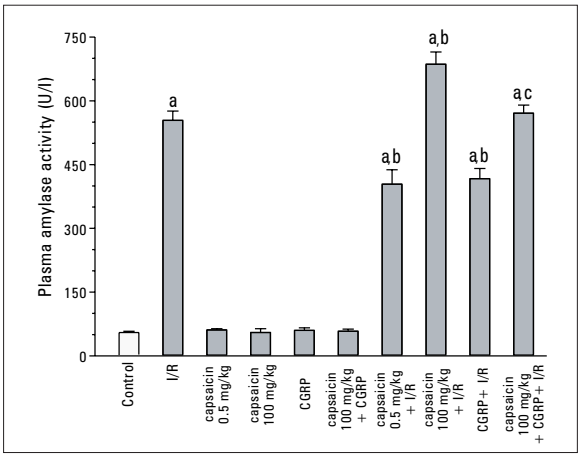

Figure 4. Effect of ischemia/reperfusion (I/R), stimulatory (0.5 mg/kg) or neurotoxic (100 mg/kg) dose of capsaicin and CGRP applied alone or in combination on plasma lipase activity. Mean±SEM of 8–15 observations in each group. (a) $P<0.05$ compared to control, (b) $P<0.05$ compared to I/R alone, (c) $P<0.05$ compared to I/R combined with neurotoxic dose of capsaicin (100 mg/kg).

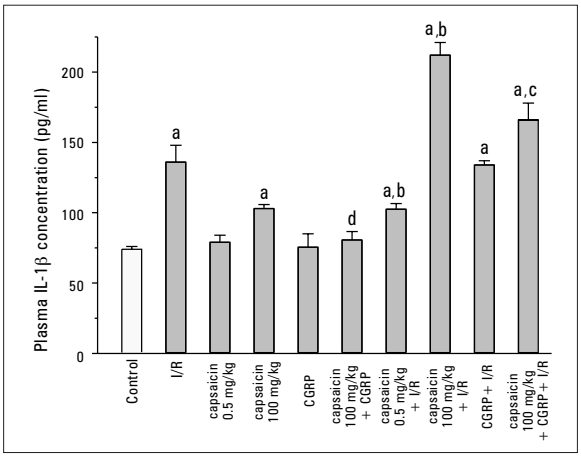

Figure 5. Effect of ischemia/reperfusion (I/R), stimulatory (0.5 mg/kg) or neurotoxic (100 mg/kg) dose of capsaicin and CGRP applied alone or in combination on plasma interleukin-1β concentration. Mean±SEM of 8–15 observations in each group. (a) $P<0.05$ compared to control, (b) $P<0.05$ compared to I/R alone, (c) $P<0.05$ compared to I/R combined with neurotoxic dose of capsaicin (100 mg/kg); (d) $P<0.05$ compared to neurotoxic dose of capsaicin (100 mg/kg) given alone.

caerulein. This procedure leads to the development of mild acute edematous pancreatitis [28]. In the present study acute pancreatitis was induced by 30 min. ischemia followed by 1 h reperfusion. This procedure leads to the development of acute severe necrotizing and hemorrhagic pancreatitis. Our present study has shown that low doses of capsaicin and pretreatment with CGRP attenuates pancreatic damage also in necrotizing pancreatitis. This observation suggests that the protective effect of sensory nerve stimulation and pretreatment with CGRP in the pancreas is independent of the etiology of acute pancreatitis.

The protective effect of sensory nerve stimulation by a low dose of capsaicin was manifested by a decrease in plasma amylase and lipase activity, a reduction in plasma concentration of IL-1β, and an increase in pancreatic DNA synthesis. We observed a close correlation between a decrease in biochemical signs of pancreatitis and an improvement of pancreatic blood flow, as well as a reduction in histologically assessed pancreatic damage. Stimulation of the sensory nerves before induction of acute pancreatitis decreased edema in pancreatic tissue edema and necrosis of acinar cells. Also, low doses of capsaicin inhibited the formation of foci of hemorrhages and reduced the leukocyte infiltration of pancreatic tissue.

Leukocyte inflammatory infiltration plays an important role in the development of pancreatic damage in the course of acute pancreatitis. Leukocytes adhere to the vascular endothelium of veins, forming plagues, and contribute to the injury by reducing blood flow via occlusion of microvessels [29]. It is well known that leukocyte adherence plays a pivotal role in the cascade of reperfusion injury [30]. Infiltration of pancreatic tissue by leukocytes leads to release of pro-inflammatory cytokines, such as IL-1β, interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) within the pancreas and systemically [31]. IL-1β is a well-known pro-inflammatory cytokine. It plays an essential role in the production of systemic acute phase responses and the release of other members of the pro-inflammatory cytokine cascade [32]. The study performed by Norman and al. [33] has shown that blockade of IL-1β prevents a rise in serum IL-6 and TNF-α level, and protects against pancreatic damage in the course of experimental acute pancreatitis. Also, neutropenia reduces systemic injury and mortality in experimental acute pancreatitis [34]. These observations are in agreement with our present data and partly elucidate the mechanism of pancreatic protection after stimulation of sensory nerves. Pretreatment with a low dose of capsaicin (0.5 mg/kg) reduced the leukocyte infiltration of pancreatic tissue and the production of IL-1β, leading to the reduction of morphological signs of pancreatic damage.

The primary cause of ischemia-reperfusion induced pancreatitis is the reduction in pancreatic blood flow and pancreatic ischemia. In the present study, stimulation of sensory nerves by a low dose of capsaicin increased pancreatic blood flow in the control animals and partly reversed the decrease in pancreatic blood flow in animals with I/R-induced pancreatitis. These results suggest that the protective effect of stimulation of sensory nerves is dependent, at least in part, on the improvement of pancreatic blood flow. Vasodilatation evoked by a low dose of capsaicin and an increase in pancreatic blood flow make it possible to remove active digestive enzymes and other mediators of inflammation from pancreatic tissue, leading to reduced pancreatic damage.

The present study has shown that ablation of sensory nerves by high doses of capsaicin (100 mg/kg) increases the severity of I/R-induced pancreatitis. Ablation of sen-

sory nerves in animals with induced acute pancreatitis led to maximal damage of pancreatic tissue in morphological terms, and caused an additional increase in plasma amylase and lipase activity, and plasma IL-1β concentration. Ablation of sensory nerves combined with induction of I/R pancreatitis resulted in maximal decrease in pancreatic blood flow and DNA synthesis. These data together with findings that stimulation of sensory nerves exhibits a protective effect on pancreatic tissue indicate that sensory nerves play an important role in defense mechanisms limiting the development of acute pancreatitis.. This observation is in agreement with previous studies involving a model of mild acute pancreatitis evoked by caerulein [16–18,27].

Treatment with CGRP before I/R caused a protective effect on the pancreas in I/R evoked pancreatitis similar to that observed after stimulation of sensory nerves, but the efficiency of treatment with CGRP was less pronounced. Also, administration of CGRP prior to I/R partly reversed the deleterious effect of deactivation of sensory nerves on I/R-induced pancreatitis. These findings indicate that the protective effect of sensory nerve stimulation on maintenance of pancreatic integrity is dependent on CGRP release. This is additionally supported by the observation that CGRP is a major mediator of thin, unmyelinated, capsaicin sensitive sensory nerves [35–37].

Various organs, including heart [38], brain [39], kidney [40], stomach [41] and pancreas [42], respond to brief exposure to ischemia with an increase in resistance to severe ischemia, a phenomenon called ischemic preconditioning. The protective effect of ischemic preconditioning in the pancreas involves sensory nerves, leading to reduced pancreatic damage, improved pancreatic blood flow, and less release of proinflammatory IL-1β. The same protective effect was observed in the present study after stimulation of sensory nerves.

CONCLUSIONS

Our study demonstrates that stimulation of sensory nerves and administration of CGRP protect the pancreas against damage evoked by I/R, whereas ablation of sensory nerves aggravates tissue damage in the pancreas exposed to I/R. CGRP is able to reverse the damage caused by ablation of sensory nerves. The beneficial effect of sensory nerve stimulation and CGRP administration on maintenance of pancreatic integrity is partly dependent on decreased IL-1β release and improved pancreatic blood flow.

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